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(51) International Patent Classification ⁶ : A61K 38/19, 38/00	A1	(11) International Publication Number: WO 98/23284 (43) International Publication Date: 4 June 1998 (04.06.98)
(21) International Application Number: PCT/US97/21451 (22) International Filing Date: 21 November 1997 (21.11.97) (30) Priority Data: 60/052,525 27 November 1996 (27.11.96) US (71) Applicant: IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). (72) Inventor: TROUTT, Anthony, B.; 3412 238th Street, S.W., Brier, WA 98036 (US). (74) Agent: PERKINS, Patricia, Anne; Immunex Corporation, 51 University Street, Seattle, WA 98101 (US).		(81) Designated States: AL, AM, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHOD OF REGULATING NITRIC OXIDE PRODUCTION		
(57) Abstract Methods for regulating levels of nitric oxide are disclosed. The methods utilize IL-17 receptors, which may be used in conjunction with inhibitors of IL-1 and/or TNF.		

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TITLE**METHOD OF REGULATING NITRIC OXIDE PRODUCTION****5 TECHNICAL FIELD OF THE INVENTION**

The present invention relates generally to the modulation of levels of nitric oxide, particularly in osteoarthritis.

BACKGROUND OF THE INVENTION

10 Cytokines are hormone-like molecules that regulate various aspects of an immune or inflammatory response; they exert their effects by specifically binding receptors present on cells, and transducing a signal to the cells. In addition to having beneficial effects (i.e., development of an effective immune response and control of infectious disease), cytokines have also been implicated in various autoimmune and inflammatory conditions.

15 Various cartilage associated cells (i.e., chondrocytes, synovial lining cells, endothelial cells, synovial fibroblasts and mononuclear cells that are present in a joint) can release nitric oxide (NO). This free radical serves as a front-line antimicrobial agent and also has antitumor effects. However, NO has also been implicated in several deleterious conditions, including autoimmune and inflammatory diseases and the bone destruction that occurs in osteoarthritis, which is not typically thought of as an inflammatory condition.

20 Rouvier et al. (*J. Immunol.* 150:5445; 1993) reported a novel cDNA which they termed CTLA-8, and which has since become known as Interleukin-17 (IL-17). IL-17 is 57% homologous to the predicted amino acid sequence of an open reading frame (ORF) present in Herpesvirus saimiri (HSV) referred to as HVS13 (Nicholas et al. *Viol.* 179:1 89, 1990; Albrecht et al., *J. Virol.* 66:5047;1992).

25 A novel receptor that binds IL-17 and its viral homolog, HVS13, has been cloned as described in USSN 08/620,694, filed March 21, 1996. The receptor is a Type I transmembrane protein; the mouse receptor has 864 amino acid residues, the human receptor has 866 amino acid residues. A soluble form of the receptor was found to inhibit various IL-17-mediated activities.

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SUMMARY OF THE INVENTION

Nitric oxide (NO) is a free radical that is involved in many phenomena, including the pathophysiological conditions of rheumatoid arthritis (RA) and osteoarthritis (OA). IL-17 stimulates production of NO by cartilage from individuals afflicted with OA. A soluble 35 form of IL-17R was found to inhibit various IL-17-mediated activities. Accordingly, soluble IL-17R will be useful in regulating levels of NO in a clinical setting.

DETAILED DESCRIPTION OF THE INVENTION

Nitric oxide is an intracellular signaling molecule that is involved in many physiological phenomena, including endothelium-dependent relaxation, neurotransmission and cell-mediated immune responses. As an antimicrobial agent, NO is effective against bacteria, viruses, helminths and parasites; it is also useful in the killing of tumor cells. Increased levels of NO occur in inflammatory disease (i.e., arthritis, ulcerative colitis, diabetes, Crohn's disease), and inhibitors of NO synthetases (NOS) have been used in experimental models of inflammatory disease, with varied effects (reviewed by A.O. Vladutiu in *Clinical Immunology and Immunopathology* 76:1-11; 1995).

Osteoarthritis (OA) has typically been considered a non-inflammatory disease, however, Amin et al. (*J. Exp. Med.* 182:2097; 1995) recently reported that the levels of NOS are upregulated in cartilage from OA patients. Incubation of OA-affected cartilage in serum-free medium resulted in the spontaneous release of substantial amounts of NO. Interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α) and lipopolysaccharide (LPS) augmented the nitrite release of OA-affected cartilage. Similar results were observed by Sakurai et al. (*J. Clin. Invest.* 96:2357, 1995) for rheumatoid arthritis patients.

IL-17 also upregulates release of NO from OA-affected cartilage. Moreover, inhibitors of IL-1 β and TNF- α do not inhibit the IL-17-augmented release of NO. Accordingly, inhibitors of IL-17 will be useful in regulating levels of NO. Such inhibitors will find therapeutic application in ameliorating the effects of NO in OA, as well as in other disease conditions in which this free radical plays a role (i.e., autoimmune and inflammatory disease).

A particularly preferred form of IL-17 inhibitor is soluble IL-17R, which is described in detail in USSN 08/620,694. IL-17 inhibitors may be used in conjunction with (i.e., simultaneously, separately or sequentially) inhibitors of IL-1 and TNF. Exemplary IL-1 inhibitors include soluble IL-1 receptors such as those described in U.S. Patents 5,319,071, 5,180,812 and 5,350,683, as well as a protein known as IL-1 receptor antagonist (IL-1RA; Eisenberg et al., *Nature* 343:341, 1990) and inhibitors of an enzyme that cleaves IL-1 into its biologically active form, as described in U.S. Patent 5,416,013.

Exemplary TNF inhibitors include soluble forms of TNF receptors, for example as described in U.S. Patent 5,395,760, and TNF receptor fusion proteins such as those disclosed in USSN 08/406,824 and USSN 08/651,286. In addition, certain virally-encoded proteins are known to bind TNF and act as TNF antagonists, as described in U.S. Patents 5,359,039 and 5,464,938; and inhibitors of an enzyme that cleaves TNF into its biologically active form are also known (see USSN 08/651,363 and USSN 08/655,345).

The relevant disclosures of the aforementioned patents and patent applications are incorporated by reference herein.

IL-17, HVS13 and homologous proteins

5 CTLA-8 refers to a cDNA cloned from an activated T cell hybridoma clone (Rouvier et al., *J. Immunol.* 150:5445; 1993). Northern blot analysis indicated that CTLA-8 transcription was very tissue specific. The CTLA-8 gene was found to map at chromosomal site 1a in mice, and at 2q31 in humans. Although a protein encoded by the CTLA-8 gene was never identified by Rouvier et al, the predicted amino acid sequence of
10 CTLA-8 was found to be 57% homologous to the predicted amino acid sequence of an ORF present in Herpesvirus Saimiri, HVS13. The CTLA-8 protein is referred to herein as Interleukin-17 (IL-17).

 The complete nucleotide sequence of the genome of HVS has been reported (Albrecht et al., *J. Virol.* 66:5047; 1992). Additional studies on one of the HVS open
15 reading frames (ORFs), HVS13, are described in Nicholas et al., *Virol.* 179:1 89; 1990. HVS13 is a late gene which is present in the Hind III-G fragment of HVS. Antisera developed against peptides derived from HVS13 are believed to react with a late protein (Nicholas et al., *supra*).

 As described USSN 08/462,353, a CIP of USSN 08/410,536, filed March 23,
20 1995, full length murine CTLA-8 protein and a CTLA-8/Fc fusion protein were expressed, tested, and found to act as a costimulus for the proliferation of T cells. Human IL-17 (CTLA-8) was identified by probing a human T cell library using a DNA fragment derived from degenerate PCR; homologs of IL-17 (CTLA-8) are expected to exist in other species as well. A full length HVS13 protein, as well as an HVS13/Fc fusion protein, were also
25 expressed, and found to act in a similar manner to IL-17 (CTLA-8) protein. Moreover, other species of herpesviruses are also likely to encode proteins homologous to that encoded by HVS13.

Proteins and Analogs

30 USSN 08/620,694, filed March 21, 1996, discloses isolated IL-17R and homologs thereof having immunoregulatory activity. Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of IL-17R within the scope of the invention also include various structural forms of the primary protein which retain biological activity. Due to the presence
35 of ionizable amino and carboxyl groups, for example, an IL-17R protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Soluble forms of IL-17R are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the murine IL-17R is shown in SEQ ID NOs:1 and 2. Computer analysis indicated that the protein has an N-terminal signal peptide with a cleavage site between amino acid 31 and 32. Those skilled in the art will recognize that the actual cleavage site may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of the cleaved peptide is expected to be within about five amino acids on either side of the predicted cleavage site. The signal peptide is followed by a 291 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 521 amino acid cytoplasmic tail. Soluble IL-17R comprises the signal peptide and the extracellular domain (residues 1 to 322 of SEQ ID NO:1) or a fragment thereof. Alternatively, a different signal peptide can be substituted for residues 1 through 31 of SEQ ID NO:1.

The nucleotide and predicted amino acid sequence of the human IL-17R is shown in SEQ ID NOs:3 and 4. It shares many features with the murine IL-17 R. Computer analysis indicated that the protein has an N-terminal signal peptide with a cleavage site between amino acid 27 and 28. Those skilled in the art will recognize that the actual cleavage site may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of the cleaved peptide is expected to be within about five amino acids on either side of the predicted cleavage site. The signal peptide is followed by a 293 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 525 amino acid cytoplasmic tail. Soluble IL-17R comprises the signal peptide and the extracellular domain (residues 1 to 320 of SEQ ID NO:1) or a fragment thereof. Alternatively, a different signal peptide can be substituted for the native signal peptide.

Other derivatives of the IL-17R protein and homologs thereof within the scope of this invention include covalent or aggregative conjugates of the protein or its fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of IL-17R proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that

described by Hopp et al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

Soluble forms of some transmembrane proteins have been expressed as fusion proteins in which an extracellular domain of a membrane protein (cognate binding region) is joined to an immunoglobulin heavy chain constant (Fc) domain. Such fusion proteins are useful as reagents to detect their cognate proteins. They are also useful as therapeutic agents in treatment of disease. However, receptors for Fc domains are present on many cell types. Thus, when a fusion protein is formed from an Fc domain and a cognate binding region, binding to a cell may occur either through binding of the cognate binding region to its cognate protein, or through binding of the Fc domain to an Fc receptor (FcR). Such binding of the Fc domain to Fc receptors may overwhelm any binding of the cognate binding region to its cognate. Moreover, binding of Fc domains to Fc receptors induces secretion of various cytokines that are involved in upregulating various aspects of an immune or inflammatory response; such upregulation has been implicated in some of the adverse effects of therapeutic administration of certain antibodies (Krutman et al., *J. Immunol.* 145:1337, 1990; Thistlewaite et al., *Am. J. Kidney Dis.* 11:112, 1988).

Jefferis et al. (*Mol. Immunol.* 27:1237; 1990) reported that a region of an antibody referred to as the hinge region (and specifically residues 234-237 within this region) determine recognition of the antibody by human Fc receptors Fc γ RI, Fc γ RII, and Fc γ RIII. Leu₍₂₃₄₎ and Leu₍₂₃₅₎ were critical to high affinity binding of IgG₃ to Fc γ RI present on U937 cells (Canfield and Morrison, *J. Exp. Med.* 173:1483; 1991). Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). These authors observed 10-100 fold decrease in affinity of IgG for FcR when a single amino acid substitution was made at a critical residue.

A single amino acid substitution in the Fc domain of an anti-CD3 monoclonal antibody (leucine to glutamic acid at position 235) was found to result in significantly less T cell activation than unmutagenized antibody, while maintaining the immunosuppressive properties (Alegre et al., *J. Immunol.* 148:3461; 1992). Wawrzynczak et al. found that murine monoclonal antibodies that contained a single amino acid substitution at residue 235 had the same serum half-life as did native antibodies (*Mol. Immunol.* 29:221; 1992). Fc domains with reduced affinity for Fc receptors are useful in the preparation of Fc fusion proteins.

Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise leucine zipper domains preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). The leucine zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The leucine zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the leucine zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Leucine zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Leucine zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 254:539; 1991) The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a leucine zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues *a* and *d* are generally hydrophobic residues, with *d* being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical leucine zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The leucine residues at position *d* contribute large hydrophobic stabilization energies, and are important for dimer formation (Krystek et al., *Int. J. Peptide Res.*

38:229, 1991). Lovejoy et al. recently reported the synthesis of a triple-stranded α -helical bundle in which the helices run up-up-down (*Science* 259:1288, 1993). Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that
5 electrostatic interactions contribute to the stoichiometry and geometry of coiled coils.

Several studies have indicated that conservative amino acids may be substituted for individual leucine residues with minimal decrease in the ability to dimerize; multiple changes, however, usually result in loss of this ability (Landschulz et al., *Science* 243:1681, 1989; Turner and Tjian, *Science* 243:1689, 1989; Hu et al., *Science* 250:1400,
10 1990). van Heekeren et al. reported that a number of different amino residues can be substituted for the leucine residues in the leucine zipper domain of GCN4, and further found that some GCN4 proteins containing two leucine substitutions were weakly active (*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect
15 syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 1992). None of the mutations affected the ability of MVF to form a tetramer.

Recently, amino acid substitutions in the *a* and *d* residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the
20 oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, CA). When all residues at position *a* are changed to isoleucine, the leucine zipper still forms a parallel dimer. When, in addition to this change, all leucine residues at position *d* are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all amino acids
25 at position *d* with isoleucine and at position *a* with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains since the mechanism of oligomer formation is believed to be the same as that for traditional leucine zipper domains such as those described above.

Derivatives of IL-17R may also be used as immunogens, reagents in *in vitro*
30 assays, or as binding agents for affinity purification procedures. Such derivatives may also be obtained by cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. The inventive proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-
35 activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the IL-17R

or against other proteins which are similar to the IL-17R, as well as other proteins that bind IL-17R or its homologous proteins.

The present invention also includes IL-17R with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of IL-17R protein or homologs thereof having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A₁ and Z, or an amino acid other than Asn between Asn and A₁.

IL-17R protein derivatives may also be obtained by mutations of the native IL-17R or its subunits. A IL-17R mutated protein, as referred to herein, is a polypeptide homologous to a IL-17R protein but which has an amino acid sequence different from the native IL-17R because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a IL-17R peptide may be easily determined by analyzing the ability of the mutated IL-17R peptide to inhibit costimulation of T or B cells by IL-17 (CTLA-8) or homologous proteins, or to bind proteins that specifically bind IL-17R (for example, antibodies or proteins encoded by the CTLA-8 cDNA or the HVS13 ORF). Moreover, activity of IL-17R analogs, muteins or derivatives can be determined by any of the assays methods described herein. Similar mutations may be made in homologs of IL-17R, and tested in a similar manner.

Bioequivalent analogs of the inventive proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the ability of the inventive proteins to

bind their ligands in a manner substantially equivalent to that of native mIL-17R or hIL-17R. Examples of conservative substitutions include substitution of amino acids outside of the binding domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of IL-17R and homologs thereof. Additional examples include
5 substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Similarly, when a deletion or insertion strategy is adopted, the potential effect of the
10 deletion or insertion on biological activity should be considered. Subunits of the inventive proteins may be constructed by deleting terminal or internal residues or sequences. Fragments of IL-17R that bind IL-17 can be readily prepared (for example, by using restriction enzymes to delete portions of the DNA) and tested for their ability to bind IL-17. Additional guidance as to the types of mutations that can be made is provided by a
15 comparison of the sequence of IL-17R to proteins that have similar structures, as well as by performing structural analysis of the inventive proteins.

Mutations in nucleotide sequences constructed for expression of analog IL-17R must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA
20 structures such as loops or hairpins which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed mutated viral proteins screened for the desired activity.

Not all mutations in the nucleotide sequence which encodes a IL-17R protein or
25 homolog thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known
30 *E. coli* preference codons for *E. coli* expression.

Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

35 Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations

set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent Nos. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference
5 herein.

Due to code degeneracy, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Other embodiments include sequences capable of hybridizing under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight)
10 to the DNA sequences encoding IL-17R, and other sequences which are degenerate to those which encode the IL-17R. In a preferred embodiment, IL-17R analogs are at least about 70 % identical in amino acid sequence to the amino acid sequence of IL-17R proteins as set forth in SEQ ID NO:1 or SEQ ID NO:3. Similarly, analogs of IL-17R homologs are at least about 70 % identical in amino acid sequence to the amino acid sequence of the native,
15 homologous proteins. In a more preferred embodiment, analogs of IL-17R or homologs thereof are at least about 80 % identical in amino acid sequence to the native form of the inventive proteins; in a most preferred embodiment, analogs of IL-17R or homologs thereof are at least about 90 % identical in amino acid sequence to the native form of the inventive proteins.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For fragments derived from the IL-17R protein, the identity is calculated based on that portion of the IL-17R protein that is present in the fragment. Similar methods can be used to
20 analyze homologs of IL-17R.

The ability of IL-17R analogs to bind CTLA-8 can be determined by testing the ability of the analogs to inhibit IL-17 (CTLA-8) -induced T cell proliferation. Alternatively, suitable assays, for example, an enzyme immunoassay or a dot blot, employing CTLA-8 or HSV13 (or a homolog thereof which binds native IL-17R) can be used to assess the ability
25 of IL-17R analogs to bind CTLA-8. Such methods are well known in the art.

Expression of Recombinant Receptors for IL-17

The proteins of the present invention are preferably produced by recombinant DNA methods by inserting a DNA sequence encoding IL-17R protein or a homolog thereof into a
35 recombinant expression vector and expressing the DNA sequence in a recombinant microbial expression system under conditions promoting expression. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments

and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being inserted in a recombinant expression vector and expressed in a recombinant transcriptional unit.

Recombinant expression vectors include synthetic or cDNA-derived DNA
5 fragments encoding IL-17R, homologs, or bioequivalent analogs, operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of
10 transcription and translation, as described in detail below. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a
15 polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA
20 sequences encoding IL-17R or homologs which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising
25 genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived
30 from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615,
35 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p.

412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in
5 *E. coli* RR1 (ATCC 53082).

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate
10 dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for
15 selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast α -factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be
20 expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

The transcriptional and translational control sequences in expression vectors to be
25 used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for
30 expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* II site located in the viral origin of replication is
35 included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen.

Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

5 A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A preferred eukaryotic vector for expression of IL-17R DNA is referred to as pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), and includes regulatory sequences derived from SV40, human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). Other preferred vectors include pDC409 and pDC410, which are derived from pDC406. pDC410 was derived from pDC406 by substituting the EBV origin of replication with sequences encoding the SV40 large T antigen. pDC409 differs from pDC406 in that a *Bgl* II restriction site outside of the multiple cloning site has been deleted, making the *Bgl* II site within the multiple cloning site unique.

15 A useful cell line that allows for episomal replication of expression vectors, such as pDC406 and pDC409, which contain the EBV origin of replication, is CV-1/EBNA (ATCC CRL 10478). The CV-1/EBNA cell line was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter.

20 Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may express the desired protein (IL-17R or homologs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

30 Suitable host cells for expression of viral proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus* spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce viral proteins using RNAs derived from the DNA constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of IL-17R or homologs that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Recombinant IL-17R may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2 μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the viral protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* *trp1* gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for Trp⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μ g/ml adenine and 20 μ g/ml uracil. Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μ g/ml adenine and 80 μ g/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, CV-1/EBNA (ATCC CRL 10478), L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian

expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

Purification of Receptors for IL-17

Purified IL-17R, homologs, or analogs are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying IL-17R and homologs thereof. For example, a IL-17R expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a IL-17R protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the IL-17R protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand (i.e., IL-17 or HVS-13) may also be used to prepare an affinity matrix for affinity purification of IL-17R.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a IL-17R composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells
5 employed in expression of recombinant viral protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale
10 fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Protein synthesized in recombinant culture is characterized by the presence of cell
15 components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the
20 inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Administration of IL-17R Compositions

The present invention provides methods of using therapeutic compositions
25 comprising an effective amount of a protein and a suitable diluent and carrier. The use of IL-17R or homologs in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated. Such molecules can be administered separately, sequentially or simultaneously with IL-17R compositions. Particularly preferred immunoregulatory molecules are soluble IL-1 receptors, soluble TNF receptors,
30 and fusion proteins thereof.

For therapeutic use, purified protein is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, IL-17R protein compositions administered to regulate NO levels can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique.
35 Typically, a therapeutic agent will be administered in the form of a composition comprising purified IL-17R, in conjunction with physiologically acceptable carriers, excipients or

diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

Receptors for IL-17 (CTLA-8) can be administered for the purpose of regulating levels of NO. Soluble IL-17R are thus likely to be useful in treatment of osteoarthritis. The inventive receptor proteins will also be useful for prevention or treatment inflammation.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

This example illustrates the ability of IL-17R to inhibit the proliferative response of T cells to mitogens. Lymphoid organs were harvested aseptically and cell suspension was created. Splenic and lymph node T cells were isolated from the cell suspension. The purity of the resulting splenic T cell preparations was routinely >95% CD3⁺ and <1% sIgM⁺. Purified murine splenic T cells (2x10⁵/well) were cultured with either 1% PHA or 1 µg/ml Con A, and a soluble IL-17R (a soluble form of IL-17R comprising the extracellular region of IL-17R fused to the Fc region of human IgG1) was titrated into the assay. Proliferation was determined after 3 days with the addition of 1 µCi [³H]thymidine. Secretion of cytokines (Interleukin-2) was determined for murine T cells cultured for 24 hr with 1 µg/ml of Con A in the presence or absence of 10 µg/ml of IL-17R.Fc or in the presence of a control Fc protein. IL-2 production was measured by ELISA and results expressed as ng/ml IL-2 produced.

Soluble IL-17R/Fc significantly inhibited the mitogen-induced proliferation of purified murine splenic T cells in a dose dependent manner, while a control Fc had no effect on the murine T cell proliferation. Complete inhibition of mitogen induced proliferation

- was observed at a soluble IL-17R.Fc concentration of 10 µg/ml. Analysis of IL-2 production by splenic T cells activated with Con A in the presence or absence of IL-17R.Fc in the culture revealed that addition of IL-17R.Fc to the T-cell culture inhibited IL-2 production to levels 8-9-fold lower than those observed in cultures containing media alone or media plus a control Fc protein. Similar results were observed when purified human T cells were used.

EXAMPLE 2

- This example illustrates the ability of IL-17R to inhibit the production of NO by cartilage-associated cells. Articular cartilage is obtained from OA-affected patients or normal controls substantially as described in Amin et al., supra. The cartilage is cut into small (approximately 3 mm) discs, which are placed in organ culture in the presence or absence of IL-17R.Fc or in the presence of a control Fc protein. Nitric oxide production is assayed by determining the nitrite level in the medium at different time intervals, for example by using a modified Griess reaction (*Anal. Biochem.* 12b:12299; 1982). Ding et al. (*J. Immunol.* 141:2407, 1988) also describe a useful method of measuring NO in *ex vivo* organ cultures of synovium and cartilage associated cells. The IL-17R.Fc is titrated to determine an effective concentration to inhibit NO production. Other soluble forms of IL-17R are also used to regulate NO levels in this manner.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Immunex Corporation
- (ii) TITLE OF INVENTION:
- 10 (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
- 15 (A) ADDRESSEE: Immunex Corporation
(B) STREET: 51 University Street
(C) CITY: Seattle
(D) STATE: WA
(E) COUNTRY: USA
(F) ZIP: 98101
- 20 (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple PowerMacintosh
(C) OPERATING SYSTEM: Apple Operating System 7.5.5
(D) SOFTWARE: Microsoft Word for PowerMacintosh, Version 6.0.1
- 25 (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:-to be assigned-
(B) FILING DATE:
(C) CLASSIFICATION:
- 30 (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: USSN 60/052,525
(B) FILING DATE: 27 NOVEMBER 1996
(C) CLASSIFICATION:
- 35 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Perkins, Patricia Anne
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(C) REFERENCE/DOCKET NUMBER: 2623-WO
- 40 (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: (206)587-0430
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- 45 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- 50 (A) LENGTH: 3288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA to mRNA
- 55 (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Mouse
 (B) CLONE: IL-17 receptor

5

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 121..2712

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	ATG GCG ATT CGG CGC TGC TGG CCA CGG GTC GTC CCC GGG CCC GCG CTG	168					
	Met Ala Ile Arg Arg Cys Trp Pro Arg Val Val Pro Gly Pro Ala Leu						
	1 5 10 15						
20	GGA TGG CTG CTT CTG CTG CTG AAC GTT CTG GCC CCG GGC CGC GCC TCC	216					
	Gly Trp Leu Leu Leu Leu Asn Val Leu Ala Pro Gly Arg Ala Ser						
	20 25 30						
25	CCG CGC CTC CTC GAC TTC CCG GCT CCG GTC TGC GCG CAG GAG GGG CTG	264					
	Pro Arg Leu Leu Asp Phe Pro Ala Pro Val Cys Ala Gln Glu Gly Leu						
	35 40 45						
30	AGC TGC AGA GTC AAG AAT AGT ACT TGT CTG GAT GAC AGC TGG ATC CAC	312					
	Ser Cys Arg Val Lys Asn Ser Thr Cys Leu Asp Asp Ser Trp Ile His						
	50 55 60						
	CCC AAA AAC CTG ACC CCG TCT TCC CCA AAA AAC ATC TAT ATC AAT CTT	360					
	Pro Lys Asn Leu Thr Pro Ser Ser Pro Lys Asn Ile Tyr Ile Asn Leu						
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	Ser Val Ser Ser Thr Gln His Gly Glu Leu Val Pro Val Leu His Val						
	85 90 95						
40	GAG TGG ACC CTG CAG ACA GAT GCC AGC ATC CTG TAC CTC GAG GGT GCA	456					
	Glu Trp Thr Leu Gln Thr Asp Ala Ser Ile Leu Tyr Leu Glu Gly Ala						
	100 105 110						
45	GAG CTG TCC GTC CTG CAG CTG AAC ACC AAT GAG CGG CTG TGT GTC AAG	504					
	Glu Leu Ser Val Leu Gln Leu Asn Thr Asn Glu Arg Leu Cys Val Lys						
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	Phe Gln Phe Leu Ser Met Leu Gln His His Arg Lys Arg Trp Arg Phe						
	130 135 140						
	TCC TTC AGC CAC TTT GTG GTA GAT CCT GGC CAG GAG TAT GAA GTG ACT	600					
	Ser Phe Ser His Phe Val Val Asp Pro Gly Gln Glu Tyr Glu Val Thr						
55	145 150 155 160						
	GTT CAC CAC CTG CCG AAG CCC ATC CCT GAT GGG GAC CCA AAC CAC AAA	648					
	Val His His Leu Pro Lys Pro Ile Pro Asp Gly Asp Pro Asn His Lys						
	165 170 175						
60							

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	Ser Lys Ile Ile Phe Val Pro Asp Cys Glu Asp Ser Lys Met Lys Met	
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5	ACT ACC TCA TGC GTG AGC TCA GGC AGC CTT TGG GAT CCC AAC ATC ACT	744
	Thr Thr Ser Cys Val Ser Ser Gly Ser Leu Trp Asp Pro Asn Ile Thr	
	195 200 205	
10	GTG GAG ACC TTG GAC ACA CAG CAT CTG CGA GTG GAC TTC ACC CTG TGG	792
	Val Glu Thr Leu Asp Thr Gln His Leu Arg Val Asp Phe Thr Leu Trp	
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15	AAT GAA TCC ACC CCC TAC CAG GTC CTG CTG GAA AGT TTC TCC GAC TCA	840
	Asn Glu Ser Thr Pro Tyr Gln Val Leu Leu Glu Ser Phe Ser Asp Ser	
	225 230 235 240	
20	GAG AAC CAC AGC TGC TTT GAT GTC GTT AAA CAA ATA TTT GCG CCC AGG	888
	Glu Asn His Ser Cys Phe Asp Val Val Lys Gln Ile Phe Ala Pro Arg	
	245 250 255	
25	CAA GAA GAA TTC CAT CAG CGA GCT AAT GTC ACA TTC ACT CTA AGC AAG	936
	Gln Glu Glu Phe His Gln Arg Ala Asn Val Thr Phe Thr Leu Ser Lys	
	260 265 270	
30	TTT CAC TGG TGC TGC CAT CAC CAC GTG CAG GTC CAG CCC TTC TTC AGC	984
	Phe His Trp Cys Cys His His Val Gln Val Gln Pro Phe Phe Ser	
	275 280 285	
35	AGC TGC CTA AAT GAC TGT TTG AGA CAC GCT GTG ACT GTG CCC TGC CCA	1032
	Ser Cys Leu Asn Asp Cys Leu Arg His Ala Val Thr Val Pro Cys Pro	
	290 295 300	
40	GTA ATC TCA AAT ACC ACA GTT CCC AAG CCA GTT GCA GAC TAC ATT CCC	1080
	Val Ile Ser Asn Thr Thr Val Pro Lys Pro Val Ala Asp Tyr Ile Pro	
	305 310 315 320	
45	CTG TGG GTG TAT GGC CTC ATC ACA CTC ATC GCC ATT CTG CTG GTG GGA	1128
	Leu Trp Val Tyr Gly Leu Ile Thr Leu Ile Ala Ile Leu Leu Val Gly	
	325 330 335	
50	TCT GTC ATC GTG CTG ATC ATC TGT ATG ACC TGG AGG CTT TCT GGC GCC	1176
	Ser Val Ile Val Leu Ile Ile Cys Met Thr Trp Arg Leu Ser Gly Ala	
	340 345 350	
55	GAT CAA GAG AAA CAT GGT GAT GAC TCC AAA ATC AAT GGC ATC TTG CCC	1224
	Asp Gln Glu Lys His Gly Asp Asp Ser Lys Ile Asn Gly Ile Leu Pro	
	355 360 365	
60	GTA GCA GAC CTG ACT CCC CCA CCC CTG AGG CCC AGG AAG GTC TGG ATC	1272
	Val Ala Asp Leu Thr Pro Pro Pro Leu Arg Pro Arg Lys Val Trp Ile	
	370 375 380	
65	GTC TAC TCG GCC GAC CAC CCC CTC TAT GTG GAG GTG GTC CTA AAG TTC	1320
	Val Tyr Ser Ala Asp His Pro Leu Tyr Val Glu Val Val Leu Lys Phe	
	385 390 395 400	
70	GCC CAG TTC CTG ATC ACT GCC TGT GGC ACT GAA GTA GCC CTT GAC CTC	1368
	Ala Gln Phe Leu Ile Thr Ala Cys Gly Thr Glu Val Ala Leu Asp Leu	
	405 410 415	

	CTG GAA GAG CAG GTT ATC TCT GAG GTG GGG GTC ATG ACC TGG GTG AGC	1416
	Leu Glu Glu Gln Val Ile Ser Glu Val Gly Val Met Thr Trp Val Ser	
	420 425 430	
5	CGA CAG AAG CAG GAG ATG GTG GAG AGC AAC TCC AAA ATC ATC ATC CTG	1464
	Arg Gln Lys Gln Glu Met Val Glu Ser Asn Ser Lys Ile Ile Ile Leu	
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10	TGT TCC CGA GGC ACC CAA GCA AAG TGG AAA GCT ATC TTG GGT TGG GCT	1512
	Cys Ser Arg Gly Thr Gln Ala Lys Trp Lys Ala Ile Leu Gly Trp Ala	
	450 455 460	
15	GAG CCT GCT GTC CAG CTA CGG TGT GAC CAC TGG AAG CCT GCT GGG GAC	1560
	Glu Pro Ala Val Gln Leu Arg Cys Asp His Trp Lys Pro Ala Gly Asp	
	465 470 475 480	
20	CTT TTC ACT GCA GCC ATG AAC ATG ATC CTG CCA GAC TTC AAG AGG CCA	1608
	Leu Phe Thr Ala Ala Met Asn Met Ile Leu Pro Asp Phe Lys Arg Pro	
	485 490 495	
25	GCC TGC TTC GGC ACC TAC GTT GTT TGC TAC TTC AGT GGC ATC TGT AGT	1656
	Ala Cys Phe Gly Thr Tyr Val Val Cys Tyr Phe Ser Gly Ile Cys Ser	
	500 505 510	
30	GAG AGG GAT GTC CCC GAC CTC TTC AAC ATC ACC TCC AGG TAC CCA CTC	1704
	Glu Arg Asp Val Pro Asp Leu Phe Asn Ile Thr Ser Arg Tyr Pro Leu	
	515 520 525	
35	ATG GAC AGA TTT GAG GAG GTT TAC TTC CGG ATC CAG GAC CTG GAG ATG	1752
	Met Asp Arg Phe Glu Glu Val Tyr Phe Arg Ile Gln Asp Leu Glu Met	
	530 535 540	
40	TTT GAA CCC GGC CGG ATG CAC CAT GTC AGA GAG CTC ACA GGG GAC AAT	1800
	Phe Glu Pro Gly Arg Met His His Val Arg Glu Leu Thr Gly Asp Asn	
	545 550 555 560	
45	TAC CTG CAG AGC CCT AGT GGC CGG CAG CTC AAG GAG GCT GTG CTT AGG	1848
	Tyr Leu Gln Ser Pro Ser Gly Arg Gln Leu Lys Glu Ala Val Leu Arg	
	565 570 575	
50	TTC CAG GAG TGG CAA ACC CAG TGC CCC GAC TGG TTC GAG CGT GAG AAC	1896
	Phe Gln Glu Trp Gln Thr Gln Cys Pro Asp Trp Phe Glu Arg Glu Asn	
	580 585 590	
55	CTC TGC TTA GCT GAT GGC CAA GAT CTT CCC TCC CTG GAT GAA GAA GTG	1944
	Leu Cys Leu Ala Asp Gly Gln Asp Leu Pro Ser Leu Asp Glu Glu Val	
	595 600 605	
60	TTT GAA GAC CCA CTG CTG CCA CCA GGG GGA GGA ATT GTC AAA CAG CAG	1992
	Phe Glu Asp Pro Leu Leu Pro Pro Gly Gly Gly Ile Val Lys Gln Gln	
	610 615 620	
65	CCC CTG GTG CGG GAA CTC CCA TCT GAC GGC TGC CTT GTG GTA GAT GTC	2040
	Pro Leu Val Arg Glu Leu Pro Ser Asp Gly Cys Leu Val Val Asp Val	
	625 630 635 640	
70	TGT GTC AGT GAG GAA GAA AGT AGA ATG GCA AAG CTG GAC CCT CAG CTA	2088
	Cys Val Ser Glu Glu Glu Ser Arg Met Ala Lys Leu Asp Pro Gln Leu	
	645 650 655	

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	Trp	Pro	Gln	Arg	Glu	Leu	Val	Ala	His	Thr	Leu	Gln	Ser	Met	Val	Leu	
			660						665					670			
5	CCA	GCA	GAG	CAG	GTC	CCT	GCA	GCT	CAT	GTG	GTG	GAG	CCT	CTC	CAT	CTC	2184
	Pro	Ala	Glu	Gln	Val	Pro	Ala	Ala	His	Val	Val	Glu	Pro	Leu	His	Leu	
			675					680					685				
10	CCA	GAC	GGC	AGT	GGA	GCA	GCT	GCC	CAG	CTG	CCC	ATG	ACA	GAG	GAC	AGC	2232
	Pro	Asp	Gly	Ser	Gly	Ala	Gly	Ala	Gln	Leu	Pro	Met	Thr	Glu	Asp	Ser	
		690					695					700					
15	GAG	GCT	TGC	CCG	CTG	CTG	GGG	GTC	CAG	AGG	AAC	AGC	ATC	CTT	TGC	CTC	2280
	Glu	Ala	Cys	Pro	Leu	Leu	Gly	Val	Gln	Arg	Asn	Ser	Ile	Leu	Cys	Leu	
	705					710					715					720	
20	CCC	GTG	GAC	TCA	GAT	GAC	TTG	CCA	CTC	TGT	AGC	ACC	CCA	ATG	ATG	TCA	2328
	Pro	Val	Asp	Ser	Asp	Asp	Leu	Pro	Leu	Cys	Ser	Thr	Pro	Met	Met	Ser	
					725					730					735		
25	CCT	GAC	CAC	CTC	CAA	GGC	GAT	GCA	AGA	GAG	CAG	CTA	GAA	AGC	CTA	ATG	2376
	Pro	Asp	His		Gln	Gly	Asp	Ala	Arg	Glu	Gln	Leu	Glu	Ser	Leu	Met	
				740					745					750			
30	CTC	TCG	GTG	CTG	CAG	CAG	AGC	CTG	AGT	GGA	CAG	CCC	CTG	GAG	AGC	TGG	2424
	Leu	Ser	Val	Leu	Gln	Gln	Ser	Leu	Ser	Gly	Gln	Pro	Leu	Glu	Ser	Trp	
			755					760					765				
35	CCG	AGG	CCA	GAG	GTG	GTC	CTC	GAG	GGC	TGC	ACA	CCC	TCT	GAG	GAG	GAG	2472
	Pro	Arg	Pro	Glu	Val	Val	Leu	Glu	Gly	Cys	Thr	Pro	Ser	Glu	Glu	Glu	
		770					775					780					
40	CAG	CGG	CAG	TCG	GTG	CAG	TCG	GAC	CAG	GGC	TAC	ATC	TCC	AGG	AGC	TCC	2520
	Gln	Arg	Gln	Ser	Val	Gln	Ser	Asp	Gln	Gly	Tyr	Ile	Ser	Arg	Ser	Ser	
	785					790					795					800	
45	CCG	CAG	CCC	CCC	GAG	TGG	CTC	ACG	GAG	GAG	GAA	GAG	CTA	GAA	CTG	GGT	2568
	Pro	Gln	Pro	Pro	Glu	Trp	Leu	Thr	Glu	Glu	Glu	Glu	Leu	Glu	Leu	Gly	
					805					810					815		
50	GAG	CCC	GTT	GAG	TCT	CTC	TCT	CCT	GAG	GAA	CTA	CGG	AGC	CTG	AGG	AAG	2616
	Glu	Pro	Val	Glu	Ser	Leu	Ser	Pro	Glu	Glu	Leu	Arg	Ser	Leu	Arg	Lys	
				820				825						830			
55	CTC	CAG	AGG	CAG	CTT	TTC	TTC	TGG	GAG	CTC	GAG	AAG	AAC	CCT	GGC	TGG	2664
	Leu	Gln	Arg	Gln	Leu	Phe	Phe	Trp	Glu	Leu	Glu	Lys	Asn	Pro	Gly	Trp	
			835					840					845				
60	AAC	AGC	TTG	GAG	CCA	CGG	AGA	CCC	ACC	CCA	GAA	GAG	CAG	AAT	CCC	TCC	2712
	Asn	Ser	Leu	Glu	Pro	Arg	Arg	Pro	Thr	Pro	Glu	Glu	Gln	Asn	Pro	Ser	
		850					855					860					
	TAG	GCCTCCTGAG	CCTGCTACTT	AAGAGGGTGT	ATATTGTA	CTGTGTGTGC											2765
55	GTGCGTGTGT	GTGTGTGTGT	GTGTGTGTGT	GTGCGTGTGT	GTGTGTGTGT	GTGTGTGTGT											2825
	GTGTGTGTAG	TGCCCCGGCTT	AGAAATGTGA	ACATCTGAAT	CTGACATAGT	GTGTGTGTGT											2885
	TGAAGTCCCA	GCACTTGGGA	ACTGAGACTT	GATGATCTCC	TGAAGCCAGG	TGTTTCAGGGC											2945

CAGTGTGAAA ACATAGCAAG ACCTCAGAGA AATCAATGCA GACATCTTGG TACTGATCCC 3005
 TAAACACACC CCTTCCCTG ATAACCCGAC ATGAGCATCT GGTCATCATT GCACAAGAAT 3065
 5 CCACAGCCCG TTCCAGAGC TCATAGCCAA GTGTGTTGCT CATTCCCTGA ATATTTATTC 3125
 TGTACCTACT ATTCATCAGA CATTTGGAAT TCAAAAACAA GTTACATGAC ACAGCCTTAG 3185
 CCACTAAGAA GCTTAAATTT CCGTAAGGAT GTAAAATTAG CCAGGATGAA TAGAGGGCTG 3245
 10 CTGCCCTGGC TGCAGAAGAG CAGGTCGTCT CGTTCCAGTC GAC 3288

(2) INFORMATION FOR SEQ ID NO:2:

15

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 864 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 Met Ala Ile Arg Arg Cys Trp Pro Arg Val Val Pro Gly Pro Ala Leu
 1 5 10 15
 Gly Trp Leu Leu Leu Leu Asn Val Leu Ala Pro Gly Arg Ala Ser
 20 25 30
 30 Pro Arg Leu Leu Asp Phe Pro Ala Pro Val Cys Ala Gln Glu Gly Leu
 35 40 45
 Ser Cys Arg Val Lys Asn Ser Thr Cys Leu Asp Asp Ser Trp Ile His
 35 50 55 60
 Pro Lys Asn Leu Thr Pro Ser Ser Pro Lys Asn Ile Tyr Ile Asn Leu
 65 70 75 80
 40 Ser Val Ser Ser Thr Gln His Gly Glu Leu Val Pro Val Leu His Val
 85 90 95
 Glu Trp Thr Leu Gln Thr Asp Ala Ser Ile Leu Tyr Leu Glu Gly Ala
 100 105 110
 45 Glu Leu Ser Val Leu Gln Leu Asn Thr Asn Glu Arg Leu Cys Val Lys
 115 120 125
 Phe Gln Phe Leu Ser Met Leu Gln His His Arg Lys Arg Trp Arg Phe
 50 130 135 140
 Ser Phe Ser His Phe Val Val Asp Pro Gly Gln Glu Tyr Glu Val Thr
 145 150 155 160
 55 Val His His Leu Pro Lys Pro Ile Pro Asp Gly Asp Pro Asn His Lys
 165 170 175
 Ser Lys Ile Ile Phe Val Pro Asp Cys Glu Asp Ser Lys Met Lys Met
 180 185 190
 60

	Thr	Thr	Ser	Cys	Val	Ser	Ser	Gly	Ser	Leu	Trp	Asp	Pro	Asn	Ile	Thr
			195					200					205			
5	Val	Glu	Thr	Leu	Asp	Thr	Gln	His	Leu	Arg	Val	Asp	Phe	Thr	Leu	Trp
	210						215					220				
	Asn	Glu	Ser	Thr	Pro	Tyr	Gln	Val	Leu	Leu	Glu	Ser	Phe	Ser	Asp	Ser
	225					230					235				240	
10	Glu	Asn	His	Ser	Cys	Phe	Asp	Val	Val	Lys	Gln	Ile	Phe	Ala	Pro	Arg
					245					250					255	
	Gln	Glu	Glu	Phe	His	Gln	Arg	Ala	Asn	Val	Thr	Phe	Thr	Leu	Ser	Lys
				260					265					270		
15	Phe	His	Trp	Cys	Cys	His	His	His	Val	Gln	Val	Gln	Pro	Phe	Phe	Ser
			275					280					285			
	Ser	Cys	Leu	Asn	Asp	Cys	Leu	Arg	His	Ala	Val	Thr	Val	Pro	Cys	Pro
20		290					295					300				
	Val	Ile	Ser	Asn	Thr	Thr	Val	Pro	Lys	Pro	Val	Ala	Asp	Tyr	Ile	Pro
	305					310					315				320	
25	Leu	Trp	Val	Tyr	Gly	Leu	Ile	Thr	Leu	Ile	Ala	Ile	Leu	Leu	Val	Gly
					325					330					335	
	Ser	Val	Ile	Val	Leu	Ile	Ile	Cys	Met	Thr	Trp	Arg	Leu	Ser	Gly	Ala
				340					345					350		
30	Asp	Gln	Glu	Lys	His	Gly	Asp	Asp	Ser	Lys	Ile	Asn	Gly	Ile	Leu	Pro
			355					360					365			
	Val	Ala	Asp	Leu	Thr	Pro	Pro	Pro	Leu	Arg	Pro	Arg	Lys	Val	Trp	Ile
35		370					375					380				
	Val	Tyr	Ser	Ala	Asp	His	Pro	Leu	Tyr	Val	Glu	Val	Val	Leu	Lys	Phe
	385					390					395				400	
40	Ala	Gln	Phe	Leu	Ile	Thr	Ala	Cys	Gly	Thr	Glu	Val	Ala	Leu	Asp	Leu
					405					410					415	
	Leu	Glu	Glu	Gln	Val	Ile	Ser	Glu	Val	Gly	Val	Met	Thr	Trp	Val	Ser
				420					425					430		
45	Arg	Gln	Lys	Gln	Glu	Met	Val	Glu	Ser	Asn	Ser	Lys	Ile	Ile	Ile	Leu
			435					440					445			
	Cys	Ser	Arg	Gly	Thr	Gln	Ala	Lys	Trp	Lys	Ala	Ile	Leu	Gly	Trp	Ala
50		450					455					460				
	Glu	Pro	Ala	Val	Gln	Leu	Arg	Cys	Asp	His	Trp	Lys	Pro	Ala	Gly	Asp
	465					470					475				480	
55	Leu	Phe	Thr	Ala	Ala	Met	Asn	Met	Ile	Leu	Pro	Asp	Phe	Lys	Arg	Pro
					485					490					495	
	Ala	Cys	Phe	Gly	Thr	Tyr	Val	Val	Cys	Tyr	Phe	Ser	Gly	Ile	Cys	Ser
60				500					505					510		

Glu Arg Asp Val Pro Asp Leu Phe Asn Ile Thr Ser Arg Tyr Pro Leu
 515 520 525
 5 Met Asp Arg Phe Glu Glu Val Tyr Phe Arg Ile Gln Asp Leu Glu Met
 530 535 540
 Phe Glu Pro Gly Arg Met His His Val Arg Glu Leu Thr Gly Asp Asn
 545 550 555 560
 10 Tyr Leu Gln Ser Pro Ser Gly Arg Gln Leu Lys Glu Ala Val Leu Arg
 565 570 575
 Phe Gln Glu Trp Gln Thr Gln Cys Pro Asp Trp Phe Glu Arg Glu Asn
 580 585 590
 15 Leu Cys Leu Ala Asp Gly Gln Asp Leu Pro Ser Leu Asp Glu Glu Val
 595 600 605
 Phe Glu Asp Pro Leu Leu Pro Pro Gly Gly Gly Ile Val Lys Gln Gln
 610 615 620
 20 Pro Leu Val Arg Glu Leu Pro Ser Asp Gly Cys Leu Val Val Asp Val
 625 630 635 640
 25 Cys Val Ser Glu Glu Glu Ser Arg Met Ala Lys Leu Asp Pro Gln Leu
 645 650 655
 Trp Pro Gln Arg Glu Leu Val Ala His Thr Leu Gln Ser Met Val Leu
 660 665 670
 30 Pro Ala Glu Gln Val Pro Ala Ala His Val Val Glu Pro Leu His Leu
 675 680 685
 Pro Asp Gly Ser Gly Ala Ala Ala Gln Leu Pro Met Thr Glu Asp Ser
 690 695 700
 35 Glu Ala Cys Pro Leu Leu Gly Val Gln Arg Asn Ser Ile Leu Cys Leu
 705 710 715 720
 40 Pro Val Asp Ser Asp Asp Leu Pro Leu Cys Ser Thr Pro Met Met Ser
 725 730 735
 Pro Asp His Leu Gln Gly Asp Ala Arg Glu Gln Leu Glu Ser Leu Met
 740 745 750
 45 Leu Ser Val Leu Gln Gln Ser Leu Ser Gly Gln Pro Leu Glu Ser Trp
 755 760 765
 Pro Arg Pro Glu Val Val Leu Glu Gly Cys Thr Pro Ser Glu Glu Glu
 770 775 780
 50 Gln Arg Gln Ser Val Gln Ser Asp Gln Gly Tyr Ile Ser Arg Ser Ser
 785 790 795 800
 55 Pro Gln Pro Pro Glu Trp Leu Thr Glu Glu Glu Glu Leu Glu Leu Gly
 805 810 815
 Glu Pro Val Glu Ser Leu Ser Pro Glu Glu Leu Arg Ser Leu Arg Lys
 820 825 830
 60

Asn Ser Leu Glu Pro Arg Arg Pro Thr Pro Glu Glu Gln Asn Pro Ser
5 850 855 860

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- 15

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35

40 CCG TCC GCT GTC CCG GGG CCC CTG CTG GGG CTG CTC CTG CTG CTC CTG 161

GGC GTG CTG GCC CCG GGT GGC GCC TCC CTG CGA CTC CTG GAC CAC CGG 209

50

55

TCC CCA AAG GAC CTG CAG ATC CAG CTG CAC TTT GCC CAC ACC CAA CAA 353
Ser Pro Lys Asp Leu Gln Ile Gln Leu His Phe Ala His Thr Gln Gln
75 80 85

	GGA GAC CTG TTC CCC GTG GCT CAC ATC GAA TGG ACA CTG CAG ACA GAC	401
	Gly Asp Leu Phe Pro Val Ala His Ile Glu Trp Thr Leu Gln Thr Asp	
	90 95 100	
5	GCC AGC ATC CTG TAC CTC GAG GGT GCA GAG TTA TCT GTC CTG CAG CTG	449
	Ala Ser Ile Leu Tyr Leu Glu Gly Ala Glu Leu Ser Val Leu Gln Leu	
	105 110 115	
10	AAC ACC AAT GAA CGT TTG TGC GTC AGG TTT GAG TTT CTG TCC AAA CTG	497
	Asn Thr Asn Glu Arg Leu Cys Val Arg Phe Glu Phe Leu Ser Lys Leu	
	120 125 130 135	
15	AGG CAT CAC CAC AGG CGG TGG CGT TTT ACC TTC AGC CAC TTT GTG GTT	545
	Arg His His His Arg Arg Trp Arg Phe Thr Phe Ser His Phe Val Val	
	140 145 150	
20	GAC CCT GAC CAG GAA TAT GAG GTG ACC GTT CAC CAC CTG CCC AAG CCC	593
	Asp Pro Asp Gln Glu Tyr Glu Val Thr Val His His Leu Pro Lys Pro	
	155 160 165	
25	ATC CCT GAT GGG GAC CCA AAC CAC CAG TCC AAG AAT TTC CTT GTG CCT	641
	Ile Pro Asp Gly Asp Pro Asn His Gln Ser Lys Asn Phe Leu Val Pro	
	170 175 180	
30	GAC TGT GAG CAC GCC AGG ATG AAG GTA ACC ACG CCA TGC ATG AGC TCA	689
	Asp Cys Glu His Ala Arg Met Lys Val Thr Thr Pro Cys Met Ser Ser	
	185 190 195	
35	GGC AGC CTG TGG GAC CCC AAC ATC ACC GTG GAG ACC CTG GAG GCC CAC	737
	Gly Ser Leu Trp Asp Pro Asn Ile Thr Val Glu Thr Leu Glu Ala His	
	200 205 210 215	
40	CAG CTG CGT GTG AGC TTC ACC CTG TGG AAC GAA TCT ACC CAT TAC CAG	785
	Gln Leu Arg Val Ser Phe Thr Leu Trp Asn Glu Ser Thr His Tyr Gln	
	220 225 230	
45	ATC CTG CTG ACC AGT TTT CCG CAC ATG GAG AAC CAC AGT TGC TTT GAG	833
	Ile Leu Leu Thr Ser Phe Pro His Met Glu Asn His Ser Cys Phe Glu	
	235 240 245	
50	CAC ATG CAC CAC ATA CCT GCG CCC AGA CCA GAA GAG TTC CAC CAG CGA	881
	His Met His His Ile Pro Ala Pro Arg Pro Glu Glu Phe His Gln Arg	
	250 255 260	
55	TCC AAC GTC ACA CTC ACT CTA CGC AAC CTT AAA GGG TGC TGT CGC CAC	929
	Ser Asn Val Thr Leu Thr Leu Arg Asn Leu Lys Gly Cys Cys Arg His	
	265 270 275	
60	CAA GTG CAG ATC CAG CCC TTC TTC AGC AGC TGC CTC AAT GAC TGC CTC	977
	Gln Val Gln Ile Gln Pro Phe Phe Ser Ser Cys Leu Asn Asp Cys Leu	
	280 285 290 295	
65	AGA CAC TCC GCG ACT GTT TCC TGC CCA GAA ATG CCA GAC ACT CCA GAA	1025
	Arg His Ser Ala Thr Val Ser Cys Pro Glu Met Pro Asp Thr Pro Glu	
	300 305 310	
70	CCA ATT CCG GAC TAC ATG CCC CTG TGG GTG TAC TGG TTC ATC ACG GGC	1073
	Pro Ile Pro Asp Tyr Met Pro Leu Trp Val Tyr Trp Phe Ile Thr Gly	
	315 320 325	

	ATC	TCC	ATC	CTG	CTG	GTG	GGC	TCC	GTC	ATC	CTG	CTC	ATC	GTC	TGC	ATG	1121
	Ile	Ser	Ile	Leu	Leu	Val	Gly	Ser	Val	Ile	Leu	Leu	Ile	Val	Cys	Met	
			330					335					340				
5	ACC	TGG	AGG	CTA	GCT	GGG	CCT	GGA	AGT	GAA	AAA	TAC	AGT	GAT	GAC	ACC	1169
	Thr	Trp	Arg	Leu	Ala	Gly	Pro	Gly	Ser	Glu	Lys	Tyr	Ser	Asp	Asp	Thr	
		345					350					355					
10	AAA	TAC	ACC	GAT	GGC	CTG	CCT	GCG	GCT	GAC	CTG	ATC	CCC	CCA	CCG	CTG	1217
	Lys	Tyr	Thr	Asp	Gly	Leu	Pro	Ala	Ala	Asp	Leu	Ile	Pro	Pro	Pro	Leu	
	360					365					370					375	
15	AAG	CCC	AGG	AAG	GTC	TGG	ATC	ATC	TAC	TCA	GCC	GAC	CAC	CCC	CTC	TAC	1265
	Lys	Pro	Arg	Lys	Val	Trp	Ile	Ile	Tyr	Ser	Ala	Asp	His	Pro	Leu	Tyr	
					380					385					390		
20	GTG	GAC	GTG	GTC	CTG	AAA	TTC	GCC	CAG	TTC	CTG	CTC	ACC	GCC	TGC	GGC	1313
	Val	Asp	Val	Val	Leu	Lys	Phe	Ala	Gln	Phe	Leu	Leu	Thr	Ala	Cys	Gly	
				395				400						405			
25	ACG	GAA	GTG	GCC	CTG	GAC	CTG	CTG	GAA	GAG	CAG	GCC	ATC	TCG	GAG	GCA	1361
	Thr	Glu	Val	Ala	Leu	Asp	Leu	Leu	Glu	Glu	Gln	Ala	Ile	Ser	Glu	Ala	
			410				415						420				
30	GGA	GTC	ATG	ACC	TGG	GTG	GGC	CGT	CAG	AAG	CAG	GAG	ATG	GTG	GAG	AGC	1409
	Gly	Val	Met	Thr	Trp	Val	Gly	Arg	Gln	Lys	Gln	Glu	Met	Val	Glu	Ser	
		425				430						435					
35	AAC	TCT	AAG	ATC	ATC	GTC	CTG	TGC	TCC	CGC	GGC	ACG	CGC	GCC	AAG	TGG	1457
	Asn	Ser	Lys	Ile	Ile	Val	Leu	Cys	Ser	Arg	Gly	Thr	Arg	Ala	Lys	Trp	
	440					445					450					455	
40	CAG	GCG	CTC	CTG	GGC	CGG	GGG	GCG	CCT	GTG	CGG	CTG	CGC	TGC	GAC	CAC	1505
	Gln	Ala	Leu	Leu	Gly	Arg	Gly	Ala	Pro	Val	Arg	Leu	Arg	Cys	Asp	His	
				460				465						470			
45	GGA	AAG	CCC	GTG	GGG	GAC	CTG	TTC	ACT	GCA	GCC	ATG	AAC	ATG	ATC	CTC	1553
	Gly	Lys	Pro	Val	Gly	Asp	Leu	Phe	Thr	Ala	Ala	Met	Asn	Met	Ile	Leu	
			475					480					485				
50	CCG	GAC	TTC	AAG	AGG	CCA	GCC	TGC	TTC	GGC	ACC	TAC	GTA	GTC	TGC	TAC	1601
	Pro	Asp	Phe	Lys	Arg	Pro	Ala	Cys	Phe	Gly	Thr	Tyr	Val	Val	Cys	Tyr	
			490				495						500				
55	TTC	AGC	GAG	GTC	AGC	TGT	GAC	GGC	GAC	GTC	CCC	GAC	CTG	TTC	GGC	GCG	1649
	Phe	Ser	Glu	Val	Ser	Cys	Asp	Gly	Asp	Val	Pro	Asp	Leu	Phe	Gly	Ala	
		505				510						515					
60	GCG	CCG	CGG	TAC	CCG	CTC	ATG	GAC	AGG	TTC	GAG	GAG	GTG	TAC	TTC	CGC	1697
	Ala	Pro	Arg	Tyr	Pro	Leu	Met	Asp	Arg	Phe	Glu	Glu	Val	Tyr	Phe	Arg	
	520					525					530					535	
65	ATC	CAG	GAC	CTG	GAG	ATG	TTC	CAG	CCG	GGC	CGC	ATG	CAC	CGC	GTA	GGG	1745
	Ile	Gln	Asp	Leu	Glu	Met	Phe	Gln	Pro	Gly	Arg	Met	His	Arg	Val	Gly	
				540						545					550		
70	GAG	CTG	TCG	GGG	GAC	AAC	TAC	CTG	CGG	AGC	CCG	GGC	GGC	AGG	CAG	CTC	1793
	Glu	Leu	Ser	Gly	Asp	Asn	Tyr	Leu	Arg	Ser	Pro	Gly	Gly	Arg	Gln	Leu	
				555					560					565			

	CGC	GCC	GCC	CTG	GAC	AGG	TTC	CGG	GAC	TGG	CAG	GTC	CGC	TGT	CCC	GAC	1841
	Arg	Ala	Ala	Leu	Asp	Arg	Phe	Arg	Asp	Trp	Gln	Val	Arg	Cys	Pro	Asp	
		570						575					580				
5	TGG	TTC	GAA	TGT	GAG	AAC	CTC	TAC	TCA	GCA	GAT	GAC	CAG	GAT	GCC	CCG	1889
	Trp	Phe	Glu	Cys	Glu	Asn	Leu	Tyr	Ser	Ala	Asp	Asp	Gln	Asp	Ala	Pro	
	585						590					595					
10	TCC	CTG	GAC	GAA	GAG	GTG	TTT	GAG	GAG	CCA	CTG	CTG	CCT	CCG	GGA	ACC	1937
	Ser	Leu	Asp	Glu	Glu	Val	Phe	Glu	Glu	Pro	Leu	Leu	Pro	Pro	Gly	Thr	
	600					605					610				615		
15	GGC	ATC	GTG	AAG	CGG	GCG	CCC	CTG	GTG	CGC	GAG	CCT	GGC	TCC	CAG	GCC	1985
	Gly	Ile	Val	Lys	Arg	Ala	Pro	Leu	Val	Arg	Glu	Pro	Gly	Ser	Gln	Ala	
					620					625					630		
20	TGC	CTG	GCC	ATA	GAC	CCG	CTG	GTC	GGG	GAG	GAA	GGA	GGA	GCA	GCA	GTG	2033
	Cys	Leu	Ala	Ile	Asp	Pro	Leu	Val	Gly	Glu	Glu	Gly	Gly	Ala	Ala	Val	
					635				640						645		
25	GCA	AAG	CTG	GAA	CCT	CAC	CTG	CAG	CCC	CGG	GGT	CAG	CCA	GCG	CCG	CAG	2081
	Ala	Lys	Leu	Glu	Pro	His	Leu	Gln	Pro	Arg	Gly	Gln	Pro	Ala	Pro	Gln	
			650					655					660				
30	CCC	CTC	CAC	ACC	CTG	GTG	CTC	GCC	GCA	GAG	GAG	GGG	GCC	CTG	GTG	GCC	2129
	Pro	Leu	His	Thr	Leu	Val	Leu	Ala	Ala	Glu	Glu	Gly	Ala	Leu	Val	Ala	
		665					670					675					
35	GCG	GTG	GAG	CCT	GGG	CCC	CTG	GCT	GAC	GGT	GCC	GCA	GTC	CGG	CTG	GCA	2177
	Ala	Val	Glu	Pro	Gly	Pro	Leu	Ala	Asp	Gly	Ala	Ala	Val	Arg	Leu	Ala	
	680					685					690					695	
40	CTG	GCG	GGG	GAG	GGC	GAG	GCC	TGC	CCG	CTG	CTG	GGC	AGC	CCG	GGC	GCT	2225
	Leu	Ala	Gly	Glu	Gly	Glu	Ala	Cys	Pro	Leu	Leu	Gly	Ser	Pro	Gly	Ala	
					700					705					710		
45	GGG	CGA	AAT	AGC	GTC	CTC	TTC	CTC	CCC	GTG	GAC	CCC	GAG	GAC	TCG	CCC	2273
	Gly	Arg	Asn	Ser	Val	Leu	Phe	Leu	Pro	Val	Asp	Pro	Glu	Asp	Ser	Pro	
				715					720					725			
50	CTT	GGC	AGC	AGC	ACC	CCC	ATG	GCG	TCT	CCT	GAC	CTC	CTT	CCA	GAG	GAC	2321
	Leu	Gly	Ser	Ser	Thr	Pro	Met	Ala	Ser	Pro	Asp	Leu	Leu	Pro	Glu	Asp	
				730				735					740				
55	GTG	AGG	GAG	CAC	CTC	GAA	GGC	TTG	ATG	CTC	TCG	CTC	TTC	GAG	CAG	AGT	2369
	Val	Arg	Glu	His	Leu	Glu	Gly	Leu	Met	Leu	Ser	Leu	Phe	Glu	Gln	Ser	
		745					750					755					
60	CTG	AGC	TGC	CAG	GCC	CAG	GGG	GGC	TGC	AGT	AGA	CCC	GCC	ATG	GTC	CTC	2417
	Leu	Ser	Cys	Gln	Ala	Gln	Gly	Gly	Cys	Ser	Arg	Pro	Ala	Met	Val	Leu	
	760					765					770				775		
65	ACA	GAC	CCA	CAC	ACG	CCC	TAC	GAG	GAG	GAG	CAG	CGG	CAG	TCA	GTG	CAG	2465
	Thr	Asp	Pro	His	Thr	Pro	Tyr	Glu	Glu	Glu	Gln	Arg	Gln	Ser	Val	Gln	
					780					785					790		
70	TCT	GAC	CAG	GGC	TAC	ATC	TCC	AGG	AGC	TCC	CCG	CAG	CCC	CCC	GAG	GGA	2513
	Ser	Asp	Gln	Gly	Tyr	Ile	Ser	Arg	Ser	Ser	Pro	Gln	Pro	Pro	Glu	Gly	
				795				800						805			

CTC ACG GAA ATG GAG GAA GAG GAG GAA GAG GAG CAG GAC CCA GGG AAG 2561
 Leu Thr Glu Met Glu Glu Glu Glu Glu Glu Glu Gln Asp Pro Gly Lys
 810 815 820

5 CCG GCC CTG CCA CTC TCT CCC GAG GAC CTG GAG AGC CTG AGG AGC CTC 2609
 Pro Ala Leu Pro Leu Ser Pro Glu Asp Leu Glu Ser Leu Arg Ser Leu
 825 830 835

10 CAG CGG CAG CTG CTT TTC CGC CAG CTG CAG AAG AAC TCG GGC TGG GAC 2657
 Gln Arg Gln Leu Leu Phe Arg Gln Leu Gln Lys Asn Ser Gly Trp Asp
 840 845 850 855

15 ACG ATG GGG TCA GAG TCA GAG GGG CCC AGT GCA TGA GGGCGGCTCC 2703
 Thr Met Gly Ser Glu Ser Glu Gly Pro Ser Ala
 860 865

CCAGGGACCG CCCAGATCCC AGCTTTGAGA GAGGAGTGTG TGTGCACGTA TTCATCTGTG 2763
 TGTACATGTC TGCATGTGTA TATGTTCTGTG TGTGAAATGT AGGCTTTAAA ATGTAAATGT 2823
 20 CTGGATTTTA ATCCCAGGCA TCCCTCCTAA CTTTTCTTTG TGCAGCGGTC TGGTTATCGT 2883
 CTATCCCCAG GGAATCCAC ACAGCCCGCT CCCAGGAGCT AATGGTAGAG CGTCCTTGAG 2943
 25 GCTCCATTAT TCGTTCATTC AGCATTTATT GTGCACCTAC TATGTGGCGG GCATTTGGGA 3003
 TACCAAGATA AATTGCATGC GGCATGGCCC CAGCCATGAA GGAACCTAAC CGCTAGTGCC 3063
 GAGGACACGT TAAACGAACA GGATGGGCCG GGCACGGTGG CTCACGCCTG TAATCCCAGC 3123
 30 ACACTGGGAG GCCGAGGCAG GTGGATCACT CTGAGGTCAG GAGTTTGAGC CAGCCTGGCC 3183
 AACATGGTGA AACCCCGGAA TTCGAGCTCG GTACCCGGGG 3223

35 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 866 amino acids
 40 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Ala Ala Arg Ser Pro Pro Ser Ala Val Pro Gly Pro Leu Leu
 1 5 10 15
 50 Gly Leu Leu Leu Leu Leu Gly Val Leu Ala Pro Gly Gly Ala Ser
 20 25 30
 Leu Arg Leu Leu Asp His Arg Ala Leu Val Cys Ser Gln Pro Gly Leu
 35 40 45
 55 Asn Cys Thr Val Lys Asn Ser Thr Cys Leu Asp Asp Ser Trp Ile His
 50 55 60
 60 Pro Arg Asn Leu Thr Pro Ser Ser Pro Lys Asp Leu Gln Ile Gln Leu
 65 70 75 80

His Phe Ala His Thr Gln Gln Gly Asp Leu Phe Pro Val Ala His Ile
 85 90 95
 5 Glu Trp Thr Leu Gln Thr Asp Ala Ser Ile Leu Tyr Leu Glu Gly Ala
 100 105 110
 Glu Leu Ser Val Leu Gln Leu Asn Thr Asn Glu Arg Leu Cys Val Arg
 115 120 125
 10 Phe Glu Phe Leu Ser Lys Leu Arg His His His Arg Arg Trp Arg Phe
 130 135 140
 Thr Phe Ser His Phe Val Val Asp Pro Asp Gln Glu Tyr Glu Val Thr
 15 145 150 155 160
 Val His His Leu Pro Lys Pro Ile Pro Asp Gly Asp Pro Asn His Gln
 165 170 175
 20 Ser Lys Asn Phe Leu Val Pro Asp Cys Glu His Ala Arg Met Lys Val
 180 185 190
 Thr Thr Pro Cys Met Ser Ser Gly Ser Leu Trp Asp Pro Asn Ile Thr
 25 195 200 205
 Val Glu Thr Leu Glu Ala His Gln Leu Arg Val Ser Phe Thr Leu Trp
 210 215 220
 Asn Glu Ser Thr His Tyr Gln Ile Leu Leu Thr Ser Phe Pro His Met
 30 225 230 235 240
 Glu Asn His Ser Cys Phe Glu His Met His His Ile Pro Ala Pro Arg
 245 250 255
 35 Pro Glu Glu Phe His Gln Arg Ser Asn Val Thr Leu Thr Leu Arg Asn
 260 265 270
 Leu Lys Gly Cys Cys Arg His Gln Val Gln Ile Gln Pro Phe Phe Ser
 275 280 285
 40 Ser Cys Leu Asn Asp Cys Leu Arg His Ser Ala Thr Val Ser Cys Pro
 290 295 300
 Glu Met Pro Asp Thr Pro Glu Pro Ile Pro Asp Tyr Met Pro Leu Trp
 45 305 310 315 320
 Val Tyr Trp Phe Ile Thr Gly Ile Ser Ile Leu Leu Val Gly Ser Val
 325 330 335
 50 Ile Leu Leu Ile Val Cys Met Thr Trp Arg Leu Ala Gly Pro Gly Ser
 340 345 350
 Glu Lys Tyr Ser Asp Asp Thr Lys Tyr Thr Asp Gly Leu Pro Ala Ala
 355 360 365
 55 Asp Leu Ile Pro Pro Pro Leu Lys Pro Arg Lys Val Trp Ile Ile Tyr
 370 375 380
 Ser Ala Asp His Pro Leu Tyr Val Asp Val Val Leu Lys Phe Ala Gln
 60 385 390 395 400

Phe Leu Leu Thr Ala Cys Gly Thr Glu Val Ala Leu Asp Leu Leu Glu
 405 410 415
 5 Glu Gln Ala Ile Ser Glu Ala Gly Val Met Thr Trp Val Gly Arg Gln
 420 425 430
 Lys Gln Glu Met Val Glu Ser Asn Ser Lys Ile Ile Val Leu Cys Ser
 435 440 445
 10 Arg Gly Thr Arg Ala Lys Trp Gln Ala Leu Leu Gly Arg Gly Ala Pro
 450 455 460
 Val Arg Leu Arg Cys Asp His Gly Lys Pro Val Gly Asp Leu Phe Thr
 15 465 470 475 480
 Ala Ala Met Asn Met Ile Leu Pro Asp Phe Lys Arg Pro Ala Cys Phe
 485 490 495
 20 Gly Thr Tyr Val Val Cys Tyr Phe Ser Glu Val Ser Cys Asp Gly Asp
 500 505 510
 Val Pro Asp Leu Phe Gly Ala Ala Pro Arg Tyr Pro Leu Met Asp Arg
 25 515 520 525
 Phe Glu Glu Val Tyr Phe Arg Ile Gln Asp Leu Glu Met Phe Gln Pro
 530 535 540
 Gly Arg Met His Arg Val Gly Glu Leu Ser Gly Asp Asn Tyr Leu Arg
 30 545 550 555 560
 Ser Pro Gly Gly Arg Gln Leu Arg Ala Ala Leu Asp Arg Phe Arg Asp
 565 570 575
 35 Trp Gln Val Arg Cys Pro Asp Trp Phe Glu Cys Glu Asn Leu Tyr Ser
 580 585 590
 Ala Asp Asp Gln Asp Ala Pro Ser Leu Asp Glu Glu Val Phe Glu Glu
 40 595 600 605
 Pro Leu Leu Pro Pro Gly Thr Gly Ile Val Lys Arg Ala Pro Leu Val
 610 615 620
 Arg Glu Pro Gly Ser Gln Ala Cys Leu Ala Ile Asp Pro Leu Val Gly
 45 625 630 635 640
 Glu Glu Gly Gly Ala Ala Val Ala Lys Leu Glu Pro His Leu Gln Pro
 645 650 655
 50 Arg Gly Gln Pro Ala Pro Gln Pro Leu His Thr Leu Val Leu Ala Ala
 660 665 670
 Glu Glu Gly Ala Leu Val Ala Ala Val Glu Pro Gly Pro Leu Ala Asp
 55 675 680 685
 Gly Ala Ala Val Arg Leu Ala Leu Ala Gly Glu Gly Glu Ala Cys Pro
 690 695 700
 Leu Leu Gly Ser Pro Gly Ala Gly Arg Asn Ser Val Leu Phe Leu Pro
 60 705 710 715 720

	Val	Asp	Pro	Glu	Asp	Ser	Pro	Leu	Gly	Ser	Ser	Thr	Pro	Met	Ala	Ser
					725					730						735
5	Pro	Asp	Leu	Leu	Pro	Glu	Asp	Val	Arg	Glu	His	Leu	Glu	Gly	Leu	Met
				740					745					750		
	Leu	Ser	Leu	Phe	Glu	Gln	Ser	Leu	Ser	Cys	Gln	Ala	Gln	Gly	Gly	Cys
			755					760						765		
10	Ser	Arg	Pro	Ala	Met	Val	Leu	Thr	Asp	Pro	His	Thr	Pro	Tyr	Glu	Glu
		770					775					780				
	Glu	Gln	Arg	Gln	Ser	Val	Gln	Ser	Asp	Gln	Gly	Tyr	Ile	Ser	Arg	Ser
15		785				790					795					800
	Ser	Pro	Gln	Pro	Pro	Glu	Gly	Leu	Thr	Glu	Met	Glu	Glu	Glu	Glu	Glu
					805					810						815
20	Glu	Glu	Gln	Asp	Pro	Gly	Lys	Pro	Ala	Leu	Pro	Leu	Ser	Pro	Glu	Asp
				820					825					830		
	Leu	Glu	Ser	Leu	Arg	Ser	Leu	Gln	Arg	Gln	Leu	Leu	Phe	Arg	Gln	Leu
			835					840					845			
25	Gln	Lys	Asn	Ser	Gly	Trp	Asp	Thr	Met	Gly	Ser	Glu	Ser	Glu	Gly	Pro
		850					855					860				
	Ser	Ala														
30		865														

CLAIMS

We claim:

1. A method for reducing the amount of nitric oxide produced by a cartilage
5 associated cell, comprising contacting the cell with a soluble Interleukin-17 receptor (IL-17R).
2. The method according to claim 1, wherein the soluble IL-17R is selected from the group consisting of:
 - (a) a protein comprising amino acids 1 through 322 of SEQ ID NO.: 2;
 - 10 (b) a protein comprising amino acids 1 through 320 of SEQ ID NO.: 4;
 - (c) a protein having an amino acid sequence that is at least about 70% identical to the amino acid sequences of the proteins of (a) or (b), and that binds IL-17; and
 - (d) fragments of the proteins of (a), (b), or (c), that bind IL-17.
3. A composition for regulation of nitric oxide levels, comprising a soluble IL-17
15 receptor and a pharmaceutically acceptable carrier or diluent.
4. The composition according to claim 3, wherein the soluble IL-17 receptor is selected from the group consisting of:
 - (a) a protein comprising amino acids 1 through 322 of SEQ ID NO.: 2;
 - (b) a protein comprising amino acids 1 through 320 of SEQ ID NO.: 4;
 - 20 (c) a protein having an amino acid sequence that is at least about 70% identical to the amino acid sequences of the proteins of (a) or (b), and that binds IL-17; and
 - (d) fragments of the proteins of (a), (b), or (c), that bind IL-17.
5. The composition according to claim 3, further comprising an immunoregulatory molecule selected from the group consisting of a soluble Type I IL-1 receptor, a soluble
25 Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.
6. The composition according to claim 4, further comprising an immunoregulatory molecule selected from the group consisting of a soluble Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein
30 comprising an IL-1 receptor and a TNF receptor, and combinations thereof.
7. The method according to claim 1, wherein the cell is simultaneously, sequentially or separately contacted with an immunoregulatory molecule selected from the

group consisting of a soluble Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.

8. The method according to claim 2, wherein the cell is simultaneously,
5 sequentially or separately contacted with an immunoregulatory molecule selected from the group consisting of a soluble Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.

9. A method of treating osteoarthritis in an individual, comprising administering to
10 the individual an amount of soluble IL-17 receptor sufficient to reduce the level of nitric oxide produced by cartilage-associated cells, in a pharmaceutically acceptable carrier or diluent.

10. The method according to claim 9, wherein the soluble IL-17 receptor is administered simultaneously, sequentially or separately with an immunoregulatory molecule
15 selected from the group consisting of a soluble Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.

11. The method according to claim 9, wherein the soluble IL-17 receptor is selected from the group consisting of:
20 (a) a protein comprising amino acids 1 through 322 of SEQ ID NO.: 2;
(b) a protein comprising amino acids 1 through 320 of SEQ ID NO.: 4;
(c) a protein having an amino acid sequence that is at least about 70% identical to the amino acid sequences of the proteins of (a) or (b), and that binds IL-17; and
(d) fragments of the proteins of (a), (b), or (c), that bind IL-17.

12. The method according to claim 11, wherein the soluble IL-17 receptor is administered simultaneously, sequentially or separately with an immunoregulatory molecule
25 selected from the group consisting of a soluble Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.

13. A method of preparing a medication for administration to a mammal afflicted with osteoarthritis, comprising formulating a soluble IL-17 receptor in a suitable excipient
30 or carrier.

14. The method of claim 13, wherein the soluble IL-17 receptor is selected from the group consisting of:

- (a) a protein comprising amino acids 1 through 322 of SEQ ID NO.: 2;
- (b) a protein comprising amino acids 1 through 320 of SEQ ID NO.: 4;
- 5 (c) a protein having an amino acid sequence that is at least about 70% identical to the amino acid sequences of the proteins of (a) or (b), and that binds IL-17; and
- (d) fragments of the proteins of (a), (b), or (c), that bind IL-17.

15. The method according to claim 13 or claim 14, wherein the medication further comprises an immunoregulatory molecule selected from the group consisting of a soluble
10 Type I IL-1 receptor, a soluble Type II IL-1 receptor, an IL-1 receptor antagonist, a soluble TNF receptor, a fusion protein comprising an IL-1 receptor and a TNF receptor, and combinations thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21451

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/19, 38/00

US CL : 514/2, 8, 12, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

(IL-17 or CTLA) and receptor, and (treat or inhibit or reduce or alleviate) and (free radical or nitric oxide or arthritis or cartilage or inflammation)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	WO 96/29408 A1 (IMMUNEX CORPORATION) 26 SEPTEMBER 1996, see the claims and pages 1-15.	1-2, 7-12 ----- 3-6, 13-15
X --- Y	YAO et al. Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity, 1995, Vol. 3, see pages 811-821.	1-2, 7-12 ----- 3-6, 13-15
Y, P	ATTUR et al. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. Arthritis and Rheumatism, 1997, Vol. 40, No. 6, see pages 1050-1053.	3-6, 13-15

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 14 FEBRUARY 1998	Date of filing of the international search report 10 MAR 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GARNETTE D. BRAPER Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/21451

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOTZ et al. IL-17 promotes cartilage degradation. Arthritis and Rheumatism, 1996, Vol. 39, No. (9 suppl.), see page S120.	3-6, 13-15
Y,P	SPRIGGS M. Interleukin-17 and its receptor. J. Clin. Immunol., 1997, Vol. 17, No. 5, see pages 366-369.	1-15
Y	YAO e al. Complete nucleotide sequence of the mouse CTLA-8 gene. Gene, 1996, Vol. 168, No. 2, see pages 223-225.	1-2, 7-12
Y	YAO et al. Human IL-17: A novel cytokine derived from T cells. J. Immunol., 1995, Vol. 155, No. 12, see pages 5483-5486.	1-2, 7-12
A	FOSSIEZ et al. T cell Interleukin-17 induces stromal cells to produce proinflammatory ad hematopoietic cytokines. J. Exp. Med., June 1996, Vol. 183, see pages 2593-2603.	1-15